Cell Tissue Res (2011) 344:1–11
DOI 10.1007/s00441-010-1094-1

MINI REVIEW

Global daily dynamics of the pineal transcriptome

Diego M. Bustos · Michael J. Bailey · David Sugden · David A. Carter · Martin F. Rath · Morten Møller · Steven L. Coon · Joan L. Weller · David C. Klein

Received: 20 September 2010 / Accepted: 16 November 2010 / Published online: 8 February 2011

Abstract Transcriptome profiling of the pineal gland has revealed night/day differences in the expression of a major fraction of the genes active in this tissue, with two-thirds of these being nocturnal increases. A set of over 600 transcripts exhibit two-fold to >100-fold daily differences in abundance. These changes appear to be primarily attributable to adrenergic-cyclic-AMP-dependent mechanisms, which are controlled via a neural pathway that includes the suprachiasmatic nucleus, the master circadian oscillator. In addition to melatonin synthesis, night/day differences in gene expression impact genes associated with several specialized functions, including the immune/inflammation response, photo-transduction, and thyroid hormone/retinoic acid biology. The following nonspecialized cellular features are also affected: adhesion, cell cycle/cell death, cytoskeleton, DNA modification, endothelium, growth, RNA modification, small molecule biology, transcription factors, vesicle biology, signaling involving Ca^{2+}, cyclic nucleotides, phospholipids, mitogen-activated protein kinases, the Wnt signaling pathway, and protein phosphorylation.

Keywords Pineal gland · Suprachiasmatic nucleus · Circadian rhythm · Gene expression · Transcriptome · Microarray · Systems biology

D. M. Bustos
Instituto Tecnológico de Chascomús (Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús), Camino Circunvalación Laguna km 6 cc164, B7130IWA Chascomús, Argentina

M. J. Bailey
Department of Poultry Science, Texas A & M University, College Station TX 77843, USA

D. Sugden
Division of Reproduction and Endocrinology, School of Biomedical and Health Sciences, King's College London, London SE1 1UL, UK

D. A. Carter
School of Biosciences, Cardiff University, Cardiff CF10 3AX, UK

M. F. Rath · M. Møller
Department of Neuroscience and Pharmacology, Faculty of Health Sciences, University of Copenhagen, Blegdamsvei 3, 2200 Copenhagen, Denmark

S. L. Coon · J. L. Weller · D. C. Klein
Section on Neuroendocrinology, Program on Developmental Endocrinology and Genetics, The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda MD 20892, USA

D. C. Klein (✉)
NIH 49/6A82, Bethesda MD 20892, USA
e-mail: Kleind@mail.nih.gov

Introduction

Two characteristics define the pineal physiology. One is its dedication to melatonin production, and the second is its adherence to a circadian schedule (Arendt 1994). These two features reflect the primary role of the pineal gland in vertebrate physiology, viz., the photochemical transduction-translation of the night/day cycle into the hormonal signal of melatonin. In all vertebrates, high levels of melatonin occur at night; day values are about ten-fold lower. In many species, day/night changes are rapid, approaching a square wave function. Accordingly, the melatonin signal provides an indication of the relative length of night and day.

Studies on the rat pineal transcriptome have made it clear that the circadian component of pineal function is not limited to melatonin production but is pervasive, impacting a broad range of processes and functions influencing essentially all aspects of the biology of the pinealocyte (Bailey et al. 2009; Fig. 1). During the course of a day, the expression of over 600 genes changes from two-fold to >100-fold, two-thirds of which represent nocturnal increases. Such changes have been detected by using a two-point mid-day/midnight sampling schedule. A greater number of rhythmically expressed genes will be identified with more frequent sampling (Bailey et al. 2009; Rath et al. 2009) and once genes are included with statistically significant night/day differences that are less than two-fold. Accordingly, a major fraction of the genes expressed in the pineal gland clearly exhibits daily differences in expression. Moreover, in many cases, the daily changes in gene expression are obviously peculiar to the pineal gland (Figs. 2, 3). The robust, global and relatively specific nature of the daily dynamics in gene expression in this tissue is evidence that the optimal functioning of the pineal gland depends upon the precise integration of multiple 24-h rhythms in many systems, in addition to melatonin synthesis.

Melatonin-rhythm-generating system

The circadian nature of melatonin production reflects three essential elements: the melatonin biosynthetic pathway, a circadian clock that controls this pathway, and a photodetector that resets the clock and also controls output to the pineal gland (Klein et al. 1999). The organization of the melatonin-rhythm-generating system (MRGS) of mammals and submammalian vertebrates differs markedly. In fishes, amphibians, and reptiles, the melatonin-producing cell is a modified photosensory pinealocyte with a self-contained MRGS; each cell detects light and contains a circadian clock and the melatonin synthetic pathway. In birds, additional photic control is mediated by the lateral eyes, reflecting an evolutionary shift toward dependence on a centralized dominating oscillator. In mammals, the shift toward centralization appears to be complete, because the melatonin-producing pinealocyte can neither detect light nor autonomously generate a circadian rhythm in melatonin production.

Mammalian MRGS

Functional anatomy Circadian signals, which control the circadian pattern of melatonin synthesis and other aspects of pineal function, originate in the suprachiasmatic nucleus (SCN; Klein et al. 1997), the master oscillator that coordinates all circadian functions in the body (Klein et al. 1991; Moore 1999; Moore et al. 2002). The SCN is located in the hypothalamus above the optic chiasm and receives input from the eyes via a retinal hypothalamic projection (Hannibal et al. 2000; Moore and Lenn 1972). The photoreceptors that control the SCN are retinal ganglion cells expressing the photopigment melanopsin (Do et al. 2009; Provencio et al. 1998; Van Gelder 2001). Light acts to reset the clock on a daily basis, ensuring that it is synchronized with night and day; light also acts to gate output from the SCN to the pineal gland. The pathway from the SCN to the pineal gland passes through the paraventricular nucleus, down the brain stem and the spinal cord to the sympathetic intermediolateral cell column in the upper thoracic segments of the spinal cord (Fig. 4). From there, nerve fibers project to the cervical sympathetic trunk to innervate neurons in the superior cervical ganglion (SCG). Projections from the SCG course to the pineal gland via the internal carotid nerve and conarian nerves (Kappers 1965; Klein 1985; Møller and Baeres 2002).

Control of dynamic changes in the pineal transcriptome Daily changes in melatonin synthesis and pineal biology in general are controlled by the sympathetic nerve fibers that innervate the pineal gland (Fig. 5; Klein 1985; Møller and Baeres 2002). These nerves serve two functions, both of which are critical for reliable circadian function. One is to release the neurotransmitter norepinephrine (NE), which activates the pinealocyte; this occurs only at night in response to stimulatory signals from the circadian clock in the SCN. During the day, these nerve fibers play the opposite role, viz., they avidly take up circulating NE and epinephrine that enter the perivascular space of the pineal gland (Parfitt and Klein 1976). This uptake capacity maintains the integrity of the day/night melatonin signal because it prevents stimulation of the pineal gland by circulating catecholamines at inappropriate times, including during periods of stress.

NE controls pineal biology via the activation of two adrenergic receptors (Ho and Chik 1990; Klein 1985). Activation of the $beta_1$-adrenergic receptor (Adrb1) partially activates adenylate cyclase (AC) and the production of cyclic AMP; the complete activation of AC requires the coincident stimulation of the $alpha_{1b}$-adrenergic receptor (Adra1b) through a regulatory "AND" gate. This elevates the intracellular Ca_I^{++} concentration and phosphatidyl inositol production, resulting in the mobilization of protein kinase A, which in turn acts on AC to potentiate the stimulation caused by Adrb1 activation. This coincident stimulation results in the maximal elevation of cyclic AMP (Ho et al. 1988; Sugden and Klein 1983, 1984, 1987, 1988; Sugden et al. 1985; Vanecek et al. 1985).

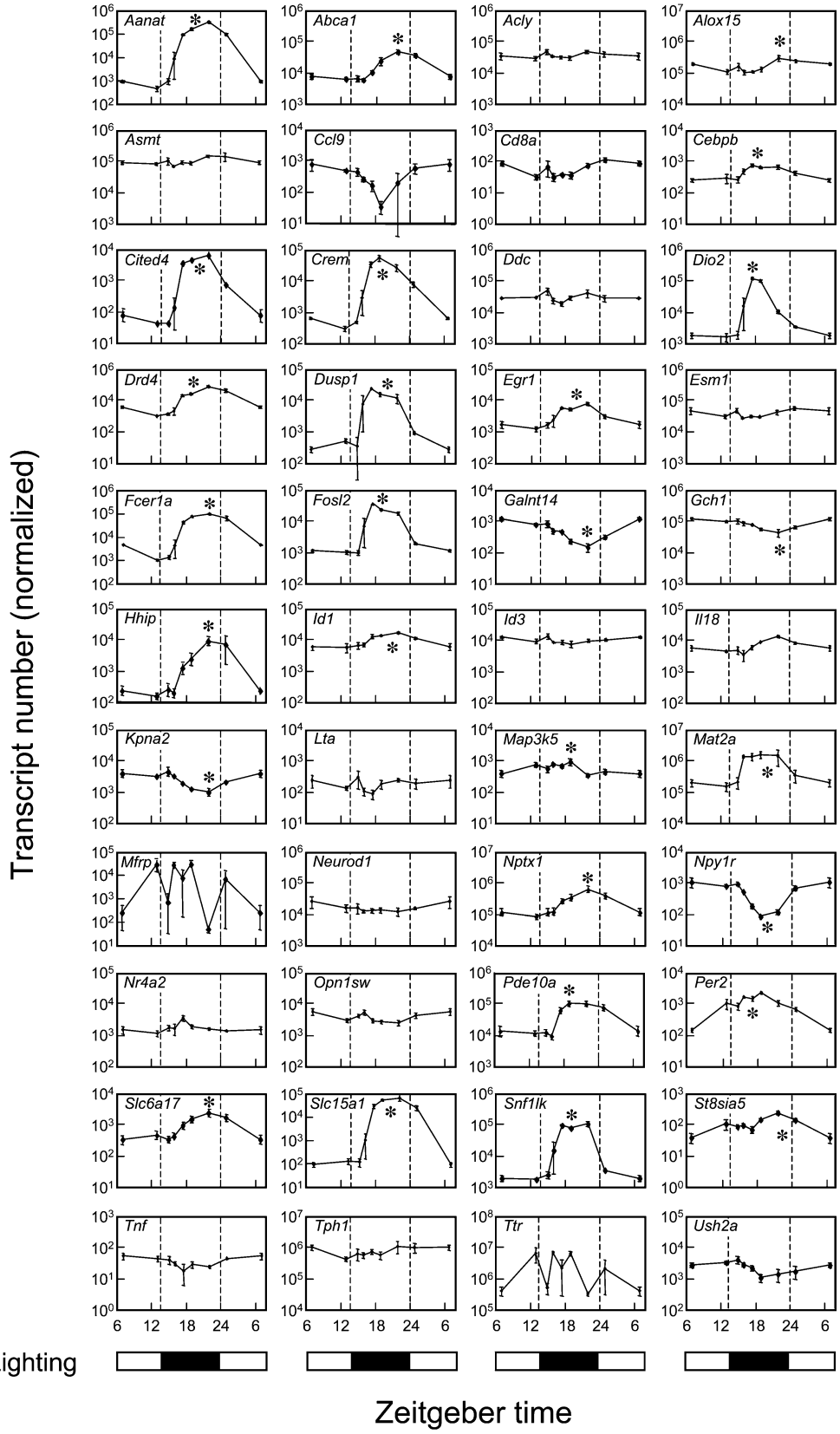


Fig. 1 Daily patterns of gene expression in the pineal gland. Entrez Gene gene symbols are used (http://www.ncbi.nlm.nih.gov/gene). Transcript number was obtained via analysis by quantitative reverse transcription with the polymerase chain reaction. The lighting cycle is represented at the *bottom* of each *column*. Each value is the mean±SE of three determinations. Values were normalized to *Actb*, *Gapdh*, *Hrpt1*, and *Rnr1*. *Statistically significant rhythmic patterns of gene expression ($P<0.01$) based on log-transformed raw values analyzed by one-way analysis of variance (for further details, see the original report of Bailey et al. 2009)

Adrenergic-cyclic-AMP control of the pineal transcriptome Global night/day changes in the pineal transcriptome occur in response to the NE-dependent increase in cyclic AMP. This is evident from the observation that treatment of pineal gland in organ culture with either NE or a cyclic AMP derivative causes nearly the same changes in gene expression that occur at night (Bailey et al. 2009).

Changes in the pineal transcriptome are a reflection of the cyclic-AMP-dependent activation of protein kinase A and the subsequent phosphorylation of the transcription factor cyclic AMP response element (CRE)-binding protein (Creb). This results in the transcription of some genes with CREs. The question of which genes are activated by this mechanism has not been fully answered. However, a cluster of other transcription factors, in addition to Creb, appear to be necessary for a gene to be expressed following Creb phosphorylation, and the tissue-specific transcriptional response to cyclic AMP seems to be determined by such clusters, each controlling a set of genes (Bailey et al. 2009).

Moreover, some genes without CREs might be indirectly regulated in response to cyclic AMP. Epigenetic events in the pinealocyte, including NE-induced changes in histone phosphorylation and acetylation, are thought to alter chromatin organization by reversing histone association with DNA, thereby permitting transcription factors access to binding sites on genes, leading to the modulation of transcription (Chik et al. 2007; Ho and Chik 2010; Ho et al. 2007; Price et al. 2009).

Rhythmically regulated functions As indicated above, the night/day differences in gene expression are seen in a broad range of cellular functions. In interpreting these changes, we should constructively consider the expression levels of all genes in a pathway in which one enzyme is rhythmically expressed. This has been performed in the case of the pineal transcriptome and has resulted in a list of "genes of interest", grouped by function (Table 1). Such a grouping and gene selection is especially useful because it identifies functionally important processes based on evidence of both dynamic and high tonic expression.

Although some of the processes identified in Table 1 can be integrated into our current knowledge of pineal biology, others cannot. In these cases, further research is required to reveal their physiological significance.

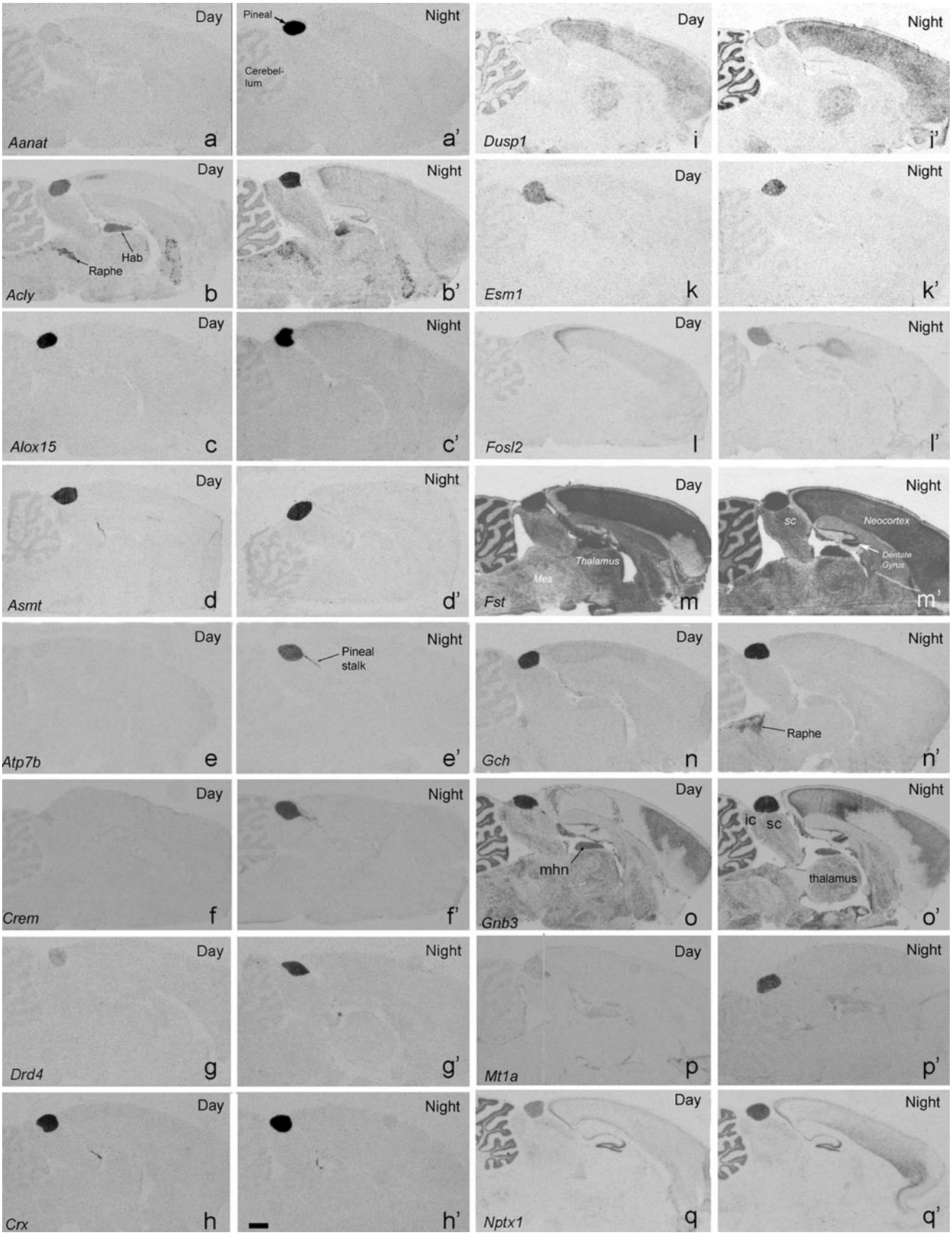
Melatonin synthesis Only one of the four genes encoding enzymes in the melatonin synthesis pathway exhibits a large daily rhythm, viz., arylalkylamine N-acetyltransferase (Aanat; Klein 2007), which converts serotonin to the melatonin precursor, N-acetylserotonin. This enzyme has been extensively studied because of its essential role in controlling melatonin synthesis. Its activity is controlled in all vertebrates through posttranslational phosphorylation; in some vertebrates, including rodents, birds, and fish, the expression of the gene is also tightly regulated. Expression increases >100-fold at night in the rat (Roseboom et al. 1996). In addition to *Aanat*, expression of the gene encoding the cofactor synthesizing enzyme *Mat2a* (methionine adenosyltransferase 2a) increases at night (Kim et al. 2005); Mat2a synthesizes the cofactor of the last enzyme in melatonin synthesis. The nocturnal increase enhances the synthetic capacity of the gland. Synthesis is low during the day because both Aanat activity and Mat2a are low. At night, the situation changes, and melatonin synthesis is limited by other factors controlling serotonin synthesis and the conversion of N-acetylserotonin to melatonin.

Analysis of the melatonin pathway is instructive, in that it clearly demonstrates the way in which a change in one element in a pathway can control an entire pathway. The other enzymes in this pathway are expressed at relatively high levels, as determined by comparison with their expression in other tissues. Specifically, in addition to Mat2a, other enzymes required for essential cofactor synthesis are highly expressed.

Immune response/inflammation A large number of rhythmically expressed or highly expressed genes in the pineal gland are associated with the immune response and inflammation, most notably a subunit of the high affinity IgE receptor, *Fcer1a* (Ganguly et al. 2007). This is a potentially important, yet poorly understood, aspect of pineal function. A hypothetical scheme has been outlined through which the pineal gland interacts with the immune system via a complex process in which pinealocytes act as sentinels for immunogens and, when appropriate, can release chemical messages that attract lymphocytes so that they can, in turn, be activated by exposure to high concentrations of pineal secretions.

Further, most mammalian pineal glands contain antigen-presenting perivascular phagocytes containing MHC Class II proteins (Møller et al. 2006). These perivascular phagocytes secrete cytokines including interleucin 1β (Tsai and McNulty 1999; Tsai et al. 2001a, 2001b). Finally, the lack of a blood-brain barrier in the mammalian pineal gland makes it possible for proteins and peptides to reach the antigen-presenting cells (Møller et al. 1978b).

Fig. 2 Tissue-specific nature of daily rhythms in gene expression in the pineal gland. Entrez Gene gene symbols are used (http://www.ncbi.nlm.nih.gov/gene). In situ hybridization autoradiographs prepared from sagittal sections of rat brains through the pineal gland. **a–q** Sections from animals killed during the day. **a'–q'** Sections from animals killed during the night. Sections were incubated with various antisense probes given *bottom left* in **a–q** (*Hab* habenula, *Mes* mesencephalon, *mhn* medial habenular nucleus, *Raphe* dorsal raphe nucleus, *sc* superior colliculus). These figures are available in high resolution at http://sne.nichd.nih.gov (for further details, see the original report of Bailey et al. 2009)


Day
Night
Aanat
a
Pineal
Cerebel-
lum
a'
Dusp1
i
i'
Hab
Raphe
Acly
b
b'
Esm1
k
k'
Alox15
c
c'
Fosl2
l
l'
Asmt
d
d'
Thalamus
Mes
Fst
m
sc
Neocortex
Dentate
Gyrus
m'
Pineal
stalk
e
e'
Gch
n
Raphe
n'
Crem
f
f'
mhn
Gnb3
o
ic
sc
thalamus
o'
Drd4
g
g'
Mt1a
p
p'
Crx
h
h'
Nptx1
q
q'

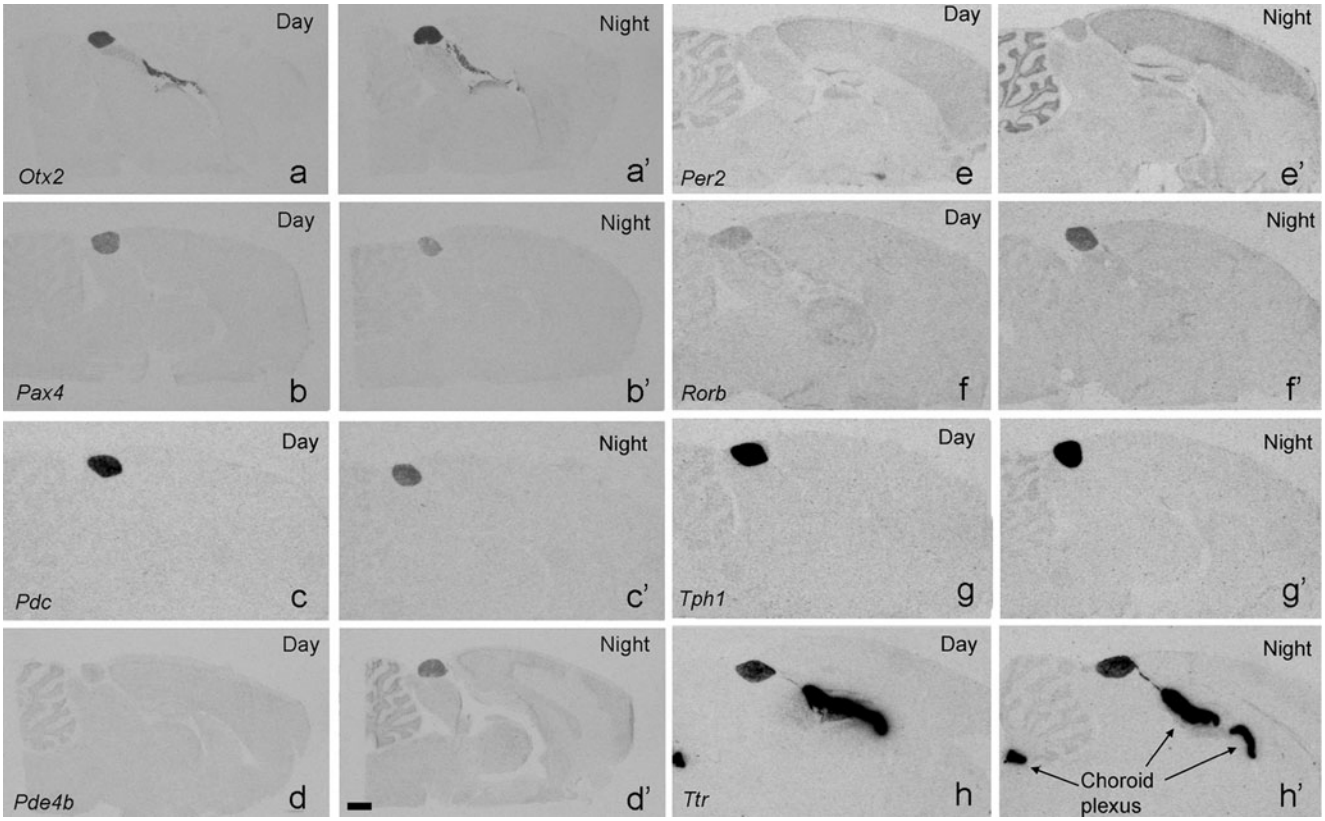


Fig. 3 Tissue-specific nature of daily rhythms in gene expression in the pineal gland. Entrez Gene gene symbols are used (http://www.ncbi.nlm.nih.gov/gene). In situ hybridization autoradiographs prepared from sagittal sections of rat brains through the pineal gland. **a–h** Sections from animals killed during the day. **a'–h'** Sections from animals killed during the night. Sections were incubated with various antisense probes given *bottom left* in **a–h**. These figures are available in high resolution at http://sne.nichd.nih.gov (for further details, see the original report of Bailey et al. 2009)

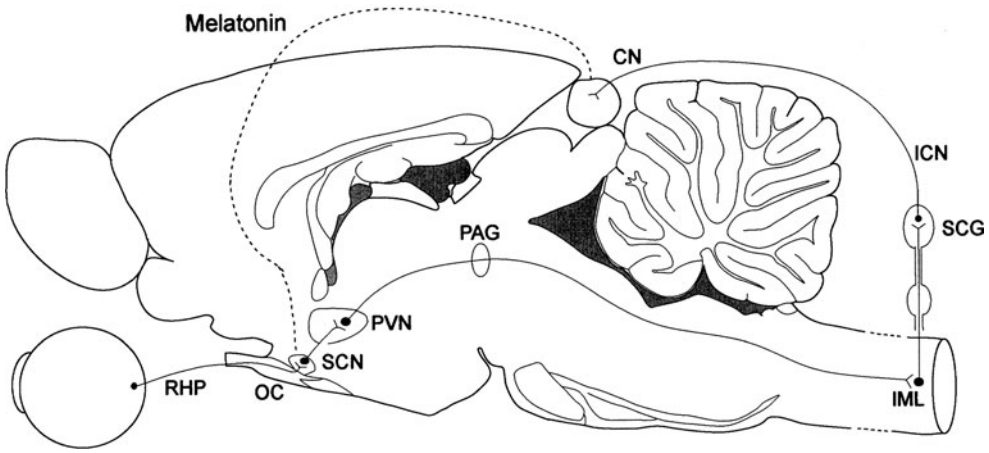


Fig. 4 Mammalian melatonin-rhythm-generating system. The circadian clock, which controls the daily rhythm in melatonin production in mammals, lies in the suprachiasmatic nucleus of the hypothalamus (*SCN*), located immediately above the optic chiasm (*OC*). SCN neurons project to the paraventricular nucleus (*PVN*), making synaptic contact with neurons of the PVN that project caudally close to the midline via the mesencephalic periaqueductal gray (*PAG*) to innervate the intermediolateral nuclei (*IML*) in the upper thoracic segments of the spinal cord. There, preganglionic neurons innervate a small subpopulation of cells that lie in the superior cervical ganglia (*SCG*) and that send projections to the pineal gland via the internal carotid nerve (*ICN*) and the conarian nerves (*CN*). At night, stimulatory signals from the SCN cause release of norepinephrine from the postganglionic nerves structures terminating in the pineal gland. Light acts on this system via the eyes through direct innervation of the SCN by the retinal hypothalamic projection (*RHP*). From Klein et al. (2010)

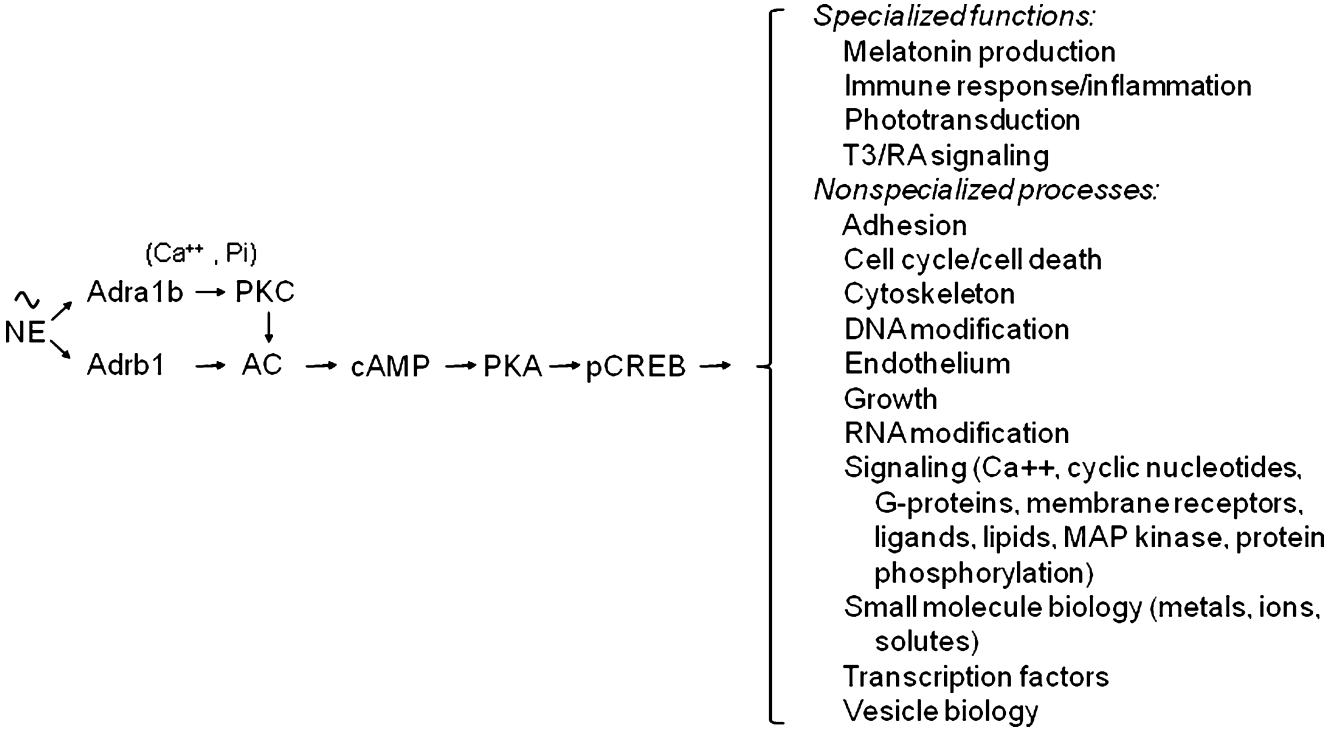


Fig. 5 Transsynaptic and cellular control of the daily dynamics of the pineal transcriptome. Sympathetic nerves release norepinephrine (*NE*), which binds to β_1-adrenergic receptors (*Adrb1*) and to α_{1b} adrenergic receptors (*Adra1b*). NE acts through the Adrb1 receptor partially activating adenylate cyclase (*AC*) and through Adra1b to potentiate the activation of AC via the Ca^{++} and phosphatidyl inositol (*Pi*) activation of protein kinase C (*PKC*). The resulting increase in cyclic AMP (*cAMP*) leads to the activation of protein kinase A (*PKA*), which phosphorylates cyclic AMP response element-binding protein (*CREB*). The consequence of this is the global shift in gene expression as seen in Figs. 1, 2, 3. The transcription factor profile unique to this tissue establishes those genes that are regulated by CREB phosphorylation. (*MAP* mitogen-activated protein)

Phototransduction-related genes The mammalian pineal gland is not directly photosensitive, although it expresses genes associated with phototransduction. This might only be a matter of evolutionary baggage, reflecting the origin of the pinealocyte and retinal photoreceptor from a common ancestral photodetector and the finding that the submammalian pineal gland is photosensitive (Klein 2004; Vigh-Teichmann et al. 1982). During early mammalian pineal ontogenesis, photoreceptive cellular elements are present but disappear before birth (Møller 1979). In the cellular context of the pinealocyte, the proteins encoded by these genes probably participate in signal transduction, as they do in the retinal photoreceptor (Lolley et al. 1992). In addition, opsins expressed in the pineal gland, including short-wave opsin *Opn1sw* (Bailey et al. 2009), might influence adrenergic receptor signaling through heterodimer formation (Franco et al. 2007).

Thyroid hormone/retinoic acid signaling A role of thyroid hormone/retinoic acid signaling in pineal function is indicated by the marked daily rhythm in genes in this pathway, including *Dio2*, *Thrb*, and *Rorb*, and the high expression of others (Bailey et al. 2009). Studies of the pineal expression of *Drd4* indicate that adrenergic-cyclic-AMP control also requires thyroid hormone, providing clear evidence of the importance of the thyroid hormone in the control of gene expression in this tissue (Kim et al. 2010).

Adhesion Daily changes in genes dedicated to cell:cell interactions and extracellular matrix biology provide clear evidence of the dynamic nature of these processes. This might impact the tightness of junctions between cells and the ability of circulating compounds and cells to gain access to the surface of pinealocytes.

Cell cycle/cell death The identification of a set of genes of interest associated with cell cycle regulation and cell death points to the presence, in the pineal gland, of one or more populations of dividing cells. These may reflect the biology of the population of the interstitial cells, which are glia-like cells with bundles of filaments immunoreactive for the glia fibrillary acid protein (Møller et al. 1978a). These genes might also be expressed in stem-cell-like undifferentiated precursor pinealocytes. Such cells have not been identified, but their presence is suggested by the expression, in the adult pineal gland, of *Pax6*, which encodes a transcription factor essential for the development of the pineal gland.

Table 1 Genes of interest in the pineal gland. Functional grouping of genes that are either highly expressed in the pineal gland more than four-fold relative to other tissues (rEx) or expressed in a rhythmic night/day pattern greater than two-fold, or both. Entrez Gene gene symbols are used (http://www.ncbi.nlm.nih.gov/gene). The most highly rhythmic and selectively expressed genes are identified as follows: * gene exhibiting a greater than four-fold night/day difference in expression; † gene exhibiting a greater than eight-fold rEx value. Modified from Bailey et al. (2009)

Specialized functions	
Melatonin synthesis	*Aanat*†, Acly, Asmt†, Ddc†, Gch1†, Gchfr, Mat2a*†, Pcbd1†, Tph1†*
Immune response/inflammation	*Abhd2[a] , And, Ahcy, Alms1[a] , ArHgef9, Bbs7, Bcar1, Btg2, C3, Ccl2†, Ccl6†, Ccl7, Ccl9†, Ccrl2[a]* , Cd1d1†, Cd47, Cd74†, Cd8a*†, Crcp†, Ctsc†, Ctss, Defb24†, Dscr1*†, Fcer1a*†, Fras1[a] , Gdf15*†, Gem[a]*†, Hivep1, Hivep2, Icsbp1, Ifi35, IfiTm1[a] , Ifnar1[a] , Igsf4a, Igsf9[a] , Igha, Il13ra2, Il17re, Il18, Il1rl1l, Ilk, Impdh2, Inhbb, Irak2*†, Irf7†, Ler3, Litaf, Lrrc8†, Lta4h, Mal2, Mdk, Mina, Mmd2, Mox2, Mx2†, Oit1[a] , Optn, Pcna, Plscr1†, Pvr, Pvrl2, RT1-A1, RT1-A2, RT1-A3, RT1-Aw2, RT1-Bb, RT1-Da, Sct2, Sema3a†, Serping1†, Slfn3, Stch, Stip1, Tfp12, Tpm4, Ush2a, Vof16†*
Photodetection	*Aipl1, Arr3, Cacna1f, Cnga1, Cngb1, Cplx3, Crocc[a], Crx, Drd4*, Egflam, Frmpd1[a], Gabrr1, Gnat2[a], Gnb3, Grk1, Guca1a[a]†, Hspa1b*, Impg1, Impg2, Kcnh6, Kcnj14, Lamp3, Lrrc21, Mak†, Mpp4, Neurod1, Opn1sw, Osap, Otx2, Pax4†, Pax6, Pcbp3, Pcdh21, Pdc, Pde6b[a] , Pla2r1[a], Rax, Rbp3, Rds, Rom1, Rtbnda, Sag, Slc24a1, Slc6a6, Slco4a1, Stk22s1, Tulp1[a], Unc119*
T3/RA signaling	*Dio2*, Hr, Rbp3, Rdh12[a] , Rorb[a]†, Rxrg†, Thrb, Ttr*
Non-specialized processes	
Adhesion	*Cdh22, Celsr32, Cml5, Cntn4, Dsc2*†, Eva[a] , Gja12[a] , Glycam1, Grn, Hnt, Mcam*†, Mfap4, Mpp4, Muc4†, Nell2, Parvb*, Pcdh21, Prph2, Pvr*†, Scarb2, Sdc4, Spon1, Ssx21p*
Cell cycle/cell death	*Acom1, Acvr1†, Aprin, Bag1, Giklk, Casp7, Ccnd2†, Cdc25a, Cdc5l, Cdk5, Cdkn1b, Cdkn1c, Cflar†, Ches1, Commd5, Csnk2a2, Ddit3*, Dnm1, Dnm2*, Elmo3, Faim, Gos2, Gadd45a, Gadd45b*, Igf1r*, Igfbpl1, Jag1, Junb, Mad2l2†, Mak10†, Ntf3, Pafah1b1, Pard3, Pdia3, Plagl1*, Ptgs2, Qscn6*†, Rarres1†, Rgc32, Fhob, Slc31a1, Strn3, Tacc3, Vegfc*
Cytoskeleton	*Ap1g1, Baiapw, Bbs4[a] , Catna1, Clasp2, Clta, Col14a1[a], Col3a1, Col4a3, Col8a1[a]†, Cope[a] , Cpg2, Dnch1, Dncl2b[a], Emilin1[a], Emls, Fgd2[a], lnb[a], Flnc[a], Fmod, Fni, Fscn2[a], Hdac11[a], †Ka15, Kif1b, Kif22, Kif2c, Krt1-18, Krt1-19†, Krt25, Lad1[a]†, Lama2[a]†, Lamb1-1[a] , Lap1b, Lcp1, Lix1†, Lmod1†, Lumk, Mapt, Marcks, Mfap5[a] , Mgp, Mrgl19, Mtap2, Mylip[a] , Nrap[a]†, Pgea1, Rpl3, Sas, Selpl[a], Sdo3, Spna2, Tctex1, Thbs4, Tmem16a[a] , Tmem22, Tpm4, Tuba4, Tubb5, Unc119, Vil2, Vim*
DNA modification	*Adprt, Blm[a] , Bnc2[a] , Cntn1, Commd1[a] ,Ctps[a] , Herc3[a] , Hmgb2, Kpna2, Mcm4, Pcna, Prc1[a] , Prim1, Ptms, Rere†, Thap4, Tlk1[a], *Top1, Tspyl4, Zdhhc22, Zfp143, Zfp162, Zfp238, Zfp36l1, Zhx1, Znf444, Zswim5[a]*
Endothelium	*Esm1†, Vegfb, Vegfc, Vwf*
Growth	*Efemt1, Egf, Egfr, Egfr1, Fgf1, Fgfr1*, Gadd45g, Gdf15*†, Gfer, Grb2, Igf1r*, Igfbp2*, Igfbp6†, Pdgfrl, Pgf, Tgfb1, Tgfbi, Vegfb, Vegfc*
RNA modification	*Ankrd24[a] , Bfsp1, Bop1, Bzw2, Eif2ak4[a] , Eif2c2, Eif3s9, Eif4g2, Ell2, Hdac5, Polr2d[a], Qtrt1, Rnase1†, Rnase2[a] , Rpat1, Sfpq, Xpot[a]**
Signaling	Calcium: *Atp2b3*, Cabp1†, Cacna1f, Cacna1g*, Calm1, Camk1g*†, Camk2b, Cip98*†, Dcamkl1, Dcamkl3*
	Cyclic nucleotide: *Adcy8*, Akap11, Cnga1, Cngb1, Creb3, Guca1a, Gucy1a3, Hcn1, Pde4b*†, Pde4d*, Pde6b, Pde8b*, Pde10a*†, Prkar2b†, Prkca*
	G-protein: *Arf3, Arr3, Arl2bp, Arl6ip5, Gem*†, Gna12, Gnaq, Gnas†, Gnat2, Gnaz, Gnb1, Gnb3, Gng11, Grk1, Pdc1, Rgs2, Rgs4, Rgs7, Rgs9, Rgs17, Sag1, Tyro3**
	Membrane receptors/ligands: *Acvr1†, Adra1b†, Adrb1†, Agtrap, Bmp6, Chrna3†, Chrnb1, Chrnb4†, Crcp†, Drd1a, Drd4*, Ece1, Ednrb, Egf, Egfr, Fgf, Fgfr1*, Fst*, Fzd4†, Grip2, Grm1*, Grm2, Hcrtr1*, Htr2c, Igf1r, Igfbp2*, Igfbp3, Igfbp5, Igfbp6†, Lepr, Nog, Opn1sw, Prlr*, Sort1, Vipr2*
	Lipid/phospholipid/cholesterol: *Abca1[a]*†, lox15, Cyp27a1†, Ephx1, Inpp5e, Itpr1, Lta4h, Ltb4dh, Pa2g1b, Pik3r3, Pla2g5†, Plcb1, Plcd4†, Ptgds, Ptgis†*
	MAP kinase: *Dusp1*, Errfi1*, Map3k5, Map3k6, Map4k1†, Mapk14, Mapk6*
	Protein phosphorylation, serine/threonine: *Calm1, Camk1g*†, Camk2b, Cdk5, Cdkn1b, Crkas, Dcamkl1, Enh, Fez1, Gsk3b, Nell2, Pak2, Prkar2b†, Prkca, Prkcdbp, Prkce, Prkcl1, Rock2*, Sik2, Snrk, Stk2, Stk39*
	Protein phosphorylation, tyrosine: *Crkas, Efna5, Jak1, Kit†, Ntrk2, Ntrk3, Ptp2E, Ptp4a1, Ptpn16, Ptprj, Ptprr, Ptp-Td14, Tyro3**
Small molecule biology	Metal homeostasis: *Atp7b*†, Chordc1, Mt1a*, Mt2, Slc30a1*, Slc39a4†*
	Ion homeostasis: *Atp1a1, Atp1b1, Atp1b2, Atp2a2, Atp2b1, Cacna1h†, Cacnb2, Clcn3, Cnga1, Cngb1, Hcn1, Kcnab2*, Kcne2†, Kcnh6, Kcnj14, Kctd3*†, Scn7a†, Slc12a2*, Slc12a5, Slc17a6*†, Slc24a1*
	Solute transport: *Slc2a1, Slc2a4, Slc3a1, Slc4a2, Slc4a4, Slc6a6, Slc7a1, Slc7a7, Slc12a2*, Slc12a5, Slc14a1, Slc15a1*†, Slc16a1, Slc16a6, Slc21a1, Slc21a7, Slc22a1, Slc25a10†, Slc29a1, Slc30a1*, Slc34a1*
Transcription factors	*Arntl, Bhlhb3, Cebpb*, Crem*†, Cbx5, Cry2*, Crx, Datf1, Eya2†, Fosl2†*, Foxd1, Hdac5, Homer1, Homer2, Hr, Isl2†, Jun, Junb, Mitf†, Msx1, Neurod1, Nr1d2, Nr1h4, Nr2f6, Nr4a1*, Nr4a3*, Otx2†, Pax4†, Pax6, Per2*, Ptch1*, Rax, Rorb, Rxrg, Thrb*
Vesicle biology	*Cadps, Chga†, Chgb, Clta, Cltb, Dnm1, Dnm2*, Dnm3, Lphn2*, Ptprn†, Scg2, Scg3, Snap23, Snap25, Sny2, Stx3, Sv2b, Syt4**

[a] Predicted gene

Pax6 is involved in the maintenance of the multipotency of retinal progenitors (Estivill-Torrus et al. 2001; Marquardt et al. 2001); it is highly expressed very early in pineal development and decreases thereafter (Rath et al. 2009).

Cytoskeleton We might reasonably suspect that daily changes in cytoskeleton biology are linked to daily changes in receptor recycling and signaling associated with the adrenergic stimulation of the pineal gland. Similarly, these changes may also be associated with the intracellular trafficking of secretory vesicles or receptor recycling.

DNA modification As described above, epigenetic regulation is a component of the cascade mediating the NE control of gene expression. Accordingly, genes associated with DNA modification are, unsurprisingly, found among the list of genes of interest.

Endothelium The endothelium in the pineal gland differs from the endothelium in the classical brain areas. In most mammals, the pineal endothelium is endowed with fenestrations (Møller et al. 1978b) making them permeable to peptides and smaller proteins. Moreover, the junctions connecting the cells are leaky to protein tracers. The high expression of endothelial genes involved in capillary growth and endothelial processes are consistent with the view that the pineal vascular bed is plastic and continually undergoing remodeling. Thus, daily changes in the endothelium may impact the ability of circulating white blood cells to enter the pineal perivascular space and to control blood flow.

Growth The set of genes of interest dedicated to the control of growth might be broadly involved in the maintenance of the pineal gland. Daily neural stimulation of the pineal gland is known to be required to maintain the normal size of the gland, and the gland becomes smaller following denervation. These changes might involve the genes associated with growth.

Signaling The large number of genes of interest dedicated to signaling includes those specifically involved in adrenergic signaling, as described above. This includes genes dedicated to the biologies of adrenergic receptors, G-proteins, Ca^{++}, phospholipids, cyclic nucleotides, MAP kinase, and protein phosphorylation. Of special interest are the rhythms in genes associated with cyclic AMP signaling (Bailey et al. 2009; for gene details, see Entrez Gene at http://www.ncbi.nlm.nih.gov/gene), including *Crem/Icer*, *Pde4B* (Kim et al. 2007), and *Snf1lk* (Kanyo et al. 2009). Within this list are genes that have no well-defined role in the pineal gland, including membrane-bound receptors and lipid-metabolizing enzymes, raising questions about their functional significance.

RNA modification Unsurprisingly, genes dedicated to RNA modification change in coordination with global changes in gene expression. Recent investigations have revealed a daily rhythm in the expression of *Mbnl2* (Kim et al. 2009). Future studies will be required to determine the precise roles that these genes play in mediating the changes that occur in gene expression.

Small molecule biology Daily changes in a number of genes encoding proteins dedicated to small molecule biology underline the importance that such molecules play in the biology of cells. These include the *Atp7b*, *Mt1a*, *Mt2*, and *Slc30a1* genes, which are dedicated to the transport and buffering of metals, and which are essential for the function of a broad range of enzymes. Others genes in this group are involved with solute transport and ion homeostasis.

Transcription factors Elucidation of the pineal transcriptome has provided a list of transcription factors, many which are not in the pineal literature. The length of this list reveals that the transcriptional regulation of the pineal transcriptome is unexpectedly complex, and that many transcription factors probably act in concert to control the pineal transcriptome. Some of the transcription-factor-encoding genes that exhibit a daily rhythm include *Crem*, *Fosl2*, *Pax4*, and *Cebpb* (Bailey et al. 2009; Rath et al. 2009). Among these, the homedomain transcription factor Pax4 peaks during the daytime in a rhythmic pattern driven by the repression of transcript levels by the nocturnal release of NE (Rath et al. 2009). In contrast, pineal expression of the Otx2 homedomain transcription factor is not influenced by the sympathetic nervous input to the gland (Rath et al. 2006).

Vesicle biology The evidence that vesicle biology is a dynamic process in the pineal gland is surprising because the main product, melatonin, is released without the involvement of vesicles. Pinealocytes contain both large dense-core granules (Møller 1976) releasing their secretory product to the interstitial space (Haldar-Misra and Pevet 1983) and smaller clear vesicles (Yatsushiro et al. 1997). Secretory vesicles in the pineal gland have received little attention, and the future purification of vesicles and characterization of their contents may reveal the presence of previously unrecognized mechanisms. The large dense-core vesicles probably contain an as yet unidentified peptide. A hint of the contents of these vesicles may be obtained from the receptor ligands that are encoded by the genes of interest and that include growth factors, Nog, Bmp4, Fst, and Wnt10a. Such vesicles might also contain glutamate, aspartate, serotonin, and gamma amino butyric acid (Mata et al. 1976; Moriyama and Yamamoto 1995).

Future directions

Our knowledge of the pineal transcriptome has been provided, to a large degree, by microarray technology. This approach is productive but limited, because it only probes a small portion of of the transcript, and because analysis is confined to those genes represented by probe sets on the available microarray chips. These problems are eliminated by high-throughput genome-wide sequencing of the transcriptome, described as RNA sequencing (RNA-Seq). This will allow a more complete documentation of the transcriptome, detailing not only those genes that are expressed, but also the relative expression of specific exons and the presence of alternative start sites and non-coding RNAs. In addition, RNA-Seq can be used to compare the expression of various genes, because sequencing is not biased, as is the case with microarray analysis.

Bioinformatics will play an important role in the construction of hypothetical regulatory cascades that control the pineal transcriptome and will lead to the testing of predictions. We anticipate that our knowledge of the pineal transcriptome will be enlarged significantly with the elucidation of the pineal microRNAome and non-coding RNAome. This will result in the construction of the control networks that integrate these molecules into the biology of the pineal cell. The dynamic nature of the pineal transcriptome presents a unique opportunity to study the way that expression is regulated, because the large physiological changes that occur represent an elegant experimental system for investigating translational control mechanisms.

References

Arendt J (1994) Melatonin and the mammalian pineal gland, 1st edn. Chapman & Hall, New York

Bailey MJ, Coon SL, Carter DA, Humphries A, Kim JS, Shi Q, Gaildrat P, Morin F, Ganguly S, Hogenesch JB et al (2009) Night/day changes in pineal expression of >600 genes: central role of adrenergic/cAMP signaling. J Biol Chem 284:7606–7622

Chik CL, Arnason TG, Dukewich WG, Price DM, Ranger A, Ho AK (2007) Histone H3 phosphorylation in the rat pineal gland: adrenergic regulation and diurnal variation. Endocrinology 148:1465–1472

Do MT, Kang SH, Xue T, Zhong H, Liao HW, Bergles DE, Yau KW (2009) Photon capture and signalling by melanopsin retinal ganglion cells. Nature 457:281–287

Estivill-Torrus G, Vitalis T, Fernandez-Llebrez P, Price DJ (2001) The transcription factor Pax6 is required for development of the diencephalic dorsal midline secretory radial glia that form the subcommissural organ. Mech Dev 109:215–224

Franco R, Casado V, Cortes A, Ferrada C, Mallol J, Woods A, Lluis C, Canela EI, Ferre S (2007) Basic concepts in G-protein-coupled receptor homo- and heterodimerization. ScientificWorldJournal 7:48–57

Ganguly S, Grodzki C, Sugden D, Møller M, Odom S, Gaildrat P, Gery I, Siraganian RP, Rivera J, Klein DC (2007) Neural adrenergic/cyclic AMP regulation of the immunoglobulin E receptor alpha-subunit expression in the mammalian pinealocyte: a neuroendocrine/immune response link? J Biol Chem 282:32758–32764

Haldar-Misra C, Pevet P (1983) The influence of different 5-methoxyindoles on the process of protein/peptide secretion characterized by the formation of granular vesicles in the mouse pineal gland. An in vitro study. Cell Tissue Res 230:113–126

Hannibal J, Møller M, Ottersen OP, Fahrenkrug J (2000) PACAP and glutamate are co-stored in the retinohypothalamic tract. J Comp Neurol 418:147–155

Ho AK, Chik CL (1990) Post-receptor mechanism in dual receptors regulation of second messengers in rat pineal gland. Prog Clin Biol Res 342:139–145

Ho AK, Chik CL (2010) Modulation of Aanat gene transcription in the rat pineal gland. J Neurochem 112:321–331

Ho AK, Price DM, Dukewich WG, Steinberg N, Arnason TG, Chik CL (2007) Acetylation of histone H3 and adrenergic-regulated gene transcription in rat pinealocytes. Endocrinology 148:4592–4600

Ho AK, Thomas TP, Chik CL, Anderson WB, Klein DC (1988) Protein kinase C: subcellular redistribution by increased Ca^{2+} influx. Evidence that Ca^{2+}-dependent subcellular redistribution of protein kinase C is involved in potentiation of beta-adrenergic stimulation of pineal cAMP and cGMP by K^{+} and A23187. J Biol Chem 263:9292–9297

Kanyo R, Price DM, Chik CL, Ho AK (2009) Salt-inducible kinase 1 in the rat pinealocyte: adrenergic regulation and role in arylalkylamine N-acetyltransferase gene transcription. Endocrinology 150:4221–4230

Kappers JA (1965) Survey of the innervation of the epiphysis cerebri and the accessory pineal organs of vertebrates. Prog Brain Res 10:87–153

Kim JS, Coon SL, Blackshaw S, Cepko CL, Møller M, Mukda S, Zhao WQ, Charlton CG, Klein DC (2005) Methionine adenosyltransferase:adrenergic-cAMP mechanism regulates a daily rhythm in pineal expression. J Biol Chem 280:677–684

Kim JS, Bailey MJ, Ho AK, Møller M, Gaildrat P, Klein DC (2007) Daily rhythm in pineal phosphodiesterase (PDE) activity reflects adrenergic/3′, 5′-cyclic adenosine 5′-monophosphate induction of the PDE4B2 variant. Endocrinology 148:1475–1485

Kim JS, Coon SL, Weller JL, Blackshaw S, Rath MF, Møller M, Klein DC (2009) Muscleblind-like 2: circadian expression in the mammalian pineal gland is controlled by an adrenergic-cAMP mechanism. J Neurochem 110:756–764

Kim JS, Bailey MJ, Weller JL, Sugden D, Rath MF, Møller M, Klein DC (2010) Thyroid hormone and adrenergic signaling interact to control pineal expression of the dopamine receptor D4 gene (Drd4). Mol Cell Endocrinol 314:128–135

Klein DC (1985) Photoneural regulation of the mammalian pineal gland. Ciba Found Symp 117:38–56

Klein DC (2004) The 2004 Aschoff/Pittendrigh lecture: theory of the origin of the pineal gland—a tale of conflict and resolution. J Biol Rhythms 19:264–279

Klein DC (2007) Arylalkylamine N-acetyltransferase: "the timezyme". J Biol Chem 282:4233–4237

Klein DC, Moore RY, Reppert SM (1991) Suprachiasmatic nucleus: the mind's clock. Oxford University Press, New York

Klein DC, Coon SL, Roseboom PH, Weller JL, Bernard M, Gastel JA, Zatz M, Iuvone PM, Rodriguez IR, Begay V et al (1997) The melatonin rhythm-generating enzyme: molecular regulation of serotonin N-acetyltransferase in the pineal gland. Recent Prog Horm Res 52:307–358

Klein DC, Baler R, Roseboom PH, Weller JL, Bernard M, Gastel JA, Zatz M, Iuvone PM, Begay V, Falcon J et al (1999) The molecular basis of the pineal melatonin rhythm: regulation of

serotonin *N*-acetylation. In: Lydic R, Baghdoyan HA (eds) Handbook of behavioral state control: cellular and molecular mechanisms. CRC Press, Boca Raton, pp 45–55

Klein DC, Bailey MJ, Carter DA, Kim JS, Shi Q, Ho AK, Chik CL, Gaildrat P, Morin F, Ganguly S et al (2010) Pineal function: impact of microarray analysis. Mol Cell Endocrinol 314:170–183

Lolley RN, Craft CM, Lee RH (1992) Photoreceptors of the retina and pinealocytes of the pineal gland share common components of signal transduction.Neurochem Res 17:81–89

Marquardt T, Ashery-Padan R, Andrejewski N, Scardigli R, Guillemot F, Gruss P (2001) Pax6 is required for the multipotent state of retinal progenitor cells. Cell 105:43–55

Mata MM, Schrier BK, Klein DC, Weller JL, Chiou CY (1976) On GABA function and physiology in the pineal gland. Brain Res 118:383–394

Møller M (1976) The ultrastructure of the human fetal pineal gland. II. Innervation and cell junctions. Cell Tissue Res 169:7–21

Møller M (1979) Presence of a pineal nerve (nervus pinealis) in fetal mammals. Prog Brain Res 52:103–106

Møller M, Baeres FM (2002) The anatomy and innervation of the mammalian pineal gland. Cell Tissue Res 309:139–150

Møller M, Ingild A, Bock E (1978a) Immunohistochemical demonstration of S-100 protein and GFA protein in interstitial cells of rat pineal gland. Brain Res 140:1–13

Møller M, Deurs B van, Westergaard E (1978b) Vascular permeability to proteins and peptides in the mouse pineal gland. Cell Tissue Res 195:1–15

Møller M, Rath MF, Klein DC (2006) The perivascular phagocyte of the mouse pineal gland: an antigen-presenting cell. Chronobiol Int 23:393–401

Moore RY (1999) A clock for the ages. Science 284:2102–2103

Moore RY, Lenn NJ (1972) A retinohypothalamic projection in the rat. J Comp Neurol 146:1–14

Moore RY, Speh JC, Leak RK (2002) Suprachiasmatic nucleus organization. Cell Tissue Res 309:89–98

Moriyama Y, Yamamoto A (1995) Vesicular L-glutamate transporter in microvesicles from bovine pineal glands. Driving force, mechanism of chloride anion activation, and substrate specificity. J Biol Chem 270:22314–22320

Parfitt AG, Klein DC (1976) Sympathetic nerve endings in the pineal gland protect against acute stress-induced increase in N-acetyltransferase (EC 2.3.1.5.) activity. Endocrinology 99:840–851

Price DM, Kanyo R, Steinberg N, Chik CL, Ho AK (2009) Nocturnal activation of aurora C in rat pineal gland: its role in the norepinephrine-induced phosphorylation of histone H3 and gene expression. Endocrinology 150:2334–2341

Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD (1998) Melanopsin: an opsin in melanophores, brain, and eye. Proc Natl Acad Sci USA 95:340–345

Rath MF, Munoz E, Ganguly S, Morin F, Shi Q, Klein DC, Møller M (2006) Expression of the Otx2 homeobox gene in the developing mammalian brain: embryonic and adult expression in the pineal gland. J Neurochem 97:556–566

Rath MF, Bailey MJ, Kim JS, Ho AK, Gaildrat P, Coon SL, Møller M, Klein DC (2009) Developmental and diurnal dynamics of Pax4 expression in the mammalian pineal gland: nocturnal down-regulation is mediated by adrenergic-cyclic adenosine 3′, 5′-monophosphate signaling. Endocrinology 150:803–811

Roseboom PH, Coon SL, Baler R, McCune SK, Weller JL, Klein DC (1996) Melatonin synthesis: analysis of the more than 150-fold nocturnal increase in serotonin N-acetyltransferase messenger ribonucleic acid in the rat pineal gland. Endocrinology 137:3033–3045

Sugden D, Klein DC (1983) Adrenergic stimulation of rat pineal hydroxyindole-O-methyltransferase. Brain Res 265:348–351

Sugden D, Klein DC (1984) Rat pineal alpha 1-adrenoceptors: identification and characterization using [125I]iodo-2-[beta-(4-hydroxyphenyl)-ethylaminomethyl]tetralone. Endocrinology 114:435–440

Sugden D, Klein DC (1987) A cholera toxin substrate regulates cyclic GMP content of rat pinealocytes. J Biol Chem 262:7447–7450

Sugden D, Klein DC (1988) Activators of protein kinase C act at a postreceptor site to amplify cyclic AMP production in rat pinealocytes. J Neurochem 50:149–155

Sugden D, Vanecek J, Klein DC, Thomas TP, Anderson WB (1985) Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes. Nature 314:359–361

Tsai SY, McNulty JA (1999) Interleukin-1beta expression in the pineal gland of the rat. J Pineal Res 27:42–48

Tsai SY, O'Brien TE, McNulty JA (2001a) Microglia play a role in mediating the effects of cytokines on the structure and function of the rat pineal gland. Cell Tissue Res 303:423–431

Tsai SY, Schluns KS, Le PT, McNulty JA (2001b) TGF-beta1 and IL-6 expression in rat pineal gland is regulated by norepinephrine and interleukin-1beta. Histol Histopathol 16:1135–1141

Van Gelder RN (2001) Non-visual ocular photoreception. Ophthalmic Genet 22:195–205

Vanecek J, Sugden D, Weller J, Klein DC (1985) Atypical synergistic alpha 1- and beta-adrenergic regulation of adenosine 3′, 5′-monophosphate and guanosine 3′, 5′-monophosphate in rat pinealocytes. Endocrinology 116:2167–2173

Vigh-Teichmann I, Korf HW, Oksche A, Vigh B (1982) Opsin-immunoreactive outer segments and acetylcholinesterase-positive neurons in the pineal complex of *Phoxinus phoxinus* (Teleostei, Cyprinidae). Cell Tissue Res 227:351–369

Yatsushiro S, Yamada H, Kozaki S, Kumon H, Michibata H, Yamamoto A, Moriyama Y (1997) L-aspartate but not the D form is secreted through microvesicle-mediated exocytosis and is sequestered through Na^+-dependent transporter in rat pinealocytes. J Neurochem 69:340–347